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PRINCIPAL INVESTIGATOR: Susan Browne, Ph.D.

CONTRACTING ORGANIZATION: Massachusetts General Hospital  
Boston, Massachusetts 02114-2696

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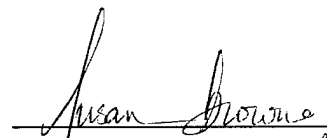
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## 5 INTRODUCTION

The overall goal of these studies is to gain insight into the roles of energy metabolism and oxidative stress in the etiology of neuronal degeneration in Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS). Using two different mouse models of HD (*Hdh* "knock-in" mice; R6/2 transgenic (Tg) mice) and one Tg mouse model of familial ALS (G93A mice overexpressing SOD1), we aim to determine the chronological order and relative contributions of bioenergetic defects and oxidative damage to cellular elements in the processes of cell death in these models. The aims of the first year of this research (October 1998-September 1999) were:

- 1.a) Measurement of local rates of cerebral glucose use ( $\text{ICMR}_{\text{glc}}$ ) in *Hdh* "knock-in" mice expressing CAG repeat lengths found in HD patients (48 CAG repeats), relative to  $\text{ICMR}_{\text{glc}}$  in wild type littermates (7 CAG repeats).
- b) Assessment of any gene dosage effect (homozygous vs. heterozygous animals).
2. Measurement of electron transport chain enzyme activities in *Hdh* knock-in mice with disease-length and normal-length CAG repeats.
3. Measurement of  $\text{ICMR}_{\text{glc}}$  in the G93A Tg mouse model of ALS. Analysis of the temporal progression of any glucose use changes by measurement at 60, 90 and 120d of age.
4. Measurement of electron transport chain activities in the G93A FALS Tg mouse model at 60 and 120d of age.

These studies concentrated on investigating parameters of energy metabolism in two mouse lines (*Hdh* and G93A), both *in vivo* and *in vitro*. Experiments used [ $^{14}\text{C}$ ]-2-deoxyglucose *in vivo* autoradiography to assess local rates of glucose metabolism in conscious, freely moving mice, and spectrophotometric assays of metabolic enzyme activities in post-mortem brain tissue.

## 6. BODY

**Objective #1:** To investigate the effect of expressing 48 CAG repeats – a CAG repeat length consistent with the generation of mutant huntingtin HD patients – on local rates of cerebral glucose use (ICMR<sub>glc</sub>) in the *Hdh* mouse model of HD; and to assess any gene dosage effect.

The pathogenetic mechanism in Huntington's disease (HD) is still unclear, although *in vivo* and *in vitro* studies in humans implicate the involvement of bioenergetic defects. Several different transgenic mouse strains expressing the huntingtin gene mutation underlying HD have been developed. Strains differ in terms of the site of mutant gene incorporation, CAG repeat length, copy number, and promotor used, and these differences are reflected in the phenotypes of the mice generated. *Hdh* CAG knock-in mice (White et al., 1997) were developed by inserting CAG repeats into exon 1 of the murine huntingtin homologue (*Hdh*) to generate a set of precise genetic HD mouse models which accurately express mutant huntingtin protein. In this study we used [<sup>14</sup>C]-2-deoxyglucose *in vivo* autoradiography in four month-old *Hdh* knock-in mice to determine: (a) if energy metabolism is altered, and (b) the effect of gene dosage on glucose use, at a time point preceding the onset of any behavioral changes and neuronal intranuclear inclusion (NII) formation in these mice.

We measured cerebral metabolic rates for glucose in 21 brain regions in *Hdh* mice both homozygous and heterozygous for the transgene, expressing 50 (*Hdh*<sup>Q50</sup>) glutamines (48/48 and 48/7 CAG repeats, respectively) and in normal wild-type littermates (*Hdh*<sup>Q7</sup>, 7/7 CAGs). Experiments were performed according to the protocol outlined in the proposal. Physiological variables (arterial plasma glucose concentration, *pO*<sub>2</sub>, *pCO*<sub>2</sub> and *pH*) were measured 35 minutes into the procedure (10 minutes before animal decapitation) to determine the physiological status of the animals (Table 1). There were no significant differences in the levels of any of these measured parameters between 7/7, 48/7 and 48/48 mice (*p* > 0.05, ANOVA, followed by Fisher's PLSD). All measured parameters were within accepted normal ranges, indicating that there are no physiological effects associated with the *Hdh* mutation which might impact on the interpretation of glucose use results.

Table 1: Physiological variables in *Hdh*<sup>Q50</sup> CAG Knock-in Mice

Variable	7/7	48/7	48/48
Arterial Glucose (mg/dL)	133.2 ± 6.0	154.3 ± 10.1	134.8 ± 6.6
<i>pO</i> <sub>2</sub> (mmHg)	92.6 ± 2.4	91.8 ± 4.2	85.5 ± 2.7
<i>pCO</i> <sub>2</sub> (mmHg)	39.7 ± 1.8	40.3 ± 1.7	39.6 ± 1.2
<i>pH</i>	7.4 ± 0.0	7.4 ± 0.0	7.4 ± 0.0

Data are mean ± SEM, variables measured 35 min after initiation of the [<sup>14</sup>C]-2-deoxyglucose procedure in *Hdh*<sup>Q7</sup> (7/7) and *Hdh*<sup>Q50</sup> (48/7 and 48/48) knock-in mice (n=4-6 per group). There were no statistically significant differences between 48/7, 48/48 and 7/7 mice in any of the parameters measured (*p*>0.05, ANOVA, followed by Fisher's PLSD).

In separate studies we have assessed glucose tolerance in *Hdh* mutant mice, in light of reports that the R6/2 tg HD mouse line is diabetic. We found that neither *Hdh* Q7, Q50, Q92 or Q111 showed any evidence of hyperglycemia or abnormal glucose handling.

We measured local cerebral metabolic rates for glucose (ICMR<sub>glc</sub>) using a modification of Sokoloff's [<sup>14</sup>C]-2-deoxyglucose *in vivo* autoradiography technique (Sokoloff et al., 1977; Browne et al., 1999). Glucose use values in each of the brain regions examined are presented in Table 2.

Table 2: Local Cerebral Glucose Utilization (ICMR<sub>glc</sub>) in *Hdh*<sup>Q50</sup> CAG Knock-in Mice

Region	7/7	48/7	48/48
Frontal Cortex	43.3 ± 2.8	45.7 ± 3.3	41.3 ± 4.7
Parietal Cortex	41.3 ± 2.5	43.8 ± 4.1	40.8 ± 4.0
Anterior Cingulate Cortex	55.0 ± 2.0	61.5 ± 6.1	56.9 ± 6.7
Auditory Cortex	50.3 ± 6.2	49.9 ± 3.8	48.7 ± 4.2
Striatum: Dorsolateral	47.5 ± 5.4	51.7 ± 5.3	46.1 ± 4.2
Ventromedial	46.0 ± 5.2	48.4 ± 4.6	45.3 ± 4.7
Globus Pallidus	29.2 ± 2.1	28.7 ± 2.5	29.2 ± 2.1
Hippocampus: CA1	27.8 ± 2.2	28.8 ± 2.6	25.7 ± 2.9
CA3	34.9 ± 2.2	37.7 ± 3.0	33.8 ± 3.9
Dentate Gyrus: Molecular Layer	45.2 ± 3.5	40.2 ± 2.8	41.7 ± 4.0
Dorsolateral Geniculate Body	42.6 ± 3.1	44.3 ± 2.9	42.7 ± 6.4
Medial Geniculate Body	46.1 ± 3.7	57.7 ± 5.3	48.7 ± 7.0
Superior Colliculus: Superficial Layer	38.2 ± 2.9	37.8 ± 2.9	36.2 ± 4.5
Deep Layer	38.5 ± 2.0	37.1 ± 2.6	37.1 ± 4.8
Internal Capsule	14.4 ± 2.0	15.5 ± 2.5	12.4 ± 2.2
Thalamus: Dorsomedial	46.1 ± 2.9	45.4 ± 4.8	44.8 ± 5.4
Ventromedial	34.9 ± 2.2	37.7 ± 3.0	33.8 ± 3.9
Substantia Nigra: <i>pars reticulata</i>	24.7 ± 1.8	27.9 ± 2.3	25.0 ± 3.6
<i>pars compacta</i>	44.6 ± 3.5	43.9 ± 3.1	40.1 ± 5.4
Cerebellum: Grey matter	28.4 ± 1.5	29.7 ± 2.6	26.8 ± 3.2
White matter	18.6 ± 1.6	19.8 ± 3.1	17.3 ± 2.0

Local cerebral metabolic rates for glucose (nmol/100g/min) in homozygous (48/48) and heterozygous (48/7) *Hdh*<sup>Q50</sup> knock-in mice, compared with rates in wild-type (7/7; *Hdh*<sup>Q7</sup>) littermate controls (n=4-6 per group). Data presented as mean ± SEM. There were no statistically significant differences between glucose use in 48/48 and 48/7 *Hdh*<sup>Q50</sup> mice and littermate wild-type controls (ANOVA, followed by Fisher's PLSD).

The [ $^{14}\text{C}$ ]-2-deoxyglucose procedure facilitates localization and quantitation of  $\text{ICMR}_{\text{glc}}$  in discrete anatomical regions throughout the CNS of conscious animals. Its utility is based on the premise that glucose is the primary energy source for cerebral cells, but brain tissue has a minimal capacity to store carbohydrate and therefore relies on glucose extraction from the circulation to fulfill energy demands. Hence, regional measurement of the rate of uptake of a radiolabelled glucose analog,  $^{14}\text{C}$ -2-deoxy-glucose, from the blood allows *in vivo* estimation of local rates of energy metabolism. Changes in glucose use generally reflect alterations in cerebral functional activity.

We found that glucose use rates did not significantly differ between *Hdh*<sup>Q50</sup> and *Hdh*<sup>Q7</sup> mice in any of the brain regions examined ( $p > 0.05$ ; ANOVA, followed by Fisher's PLSD), and there was no effect of gene dosage in *Hdh*<sup>Q50</sup> mice (48/48 vs 48/7). This contrasts with the preliminary results reported in the grant proposal which suggested that glucose use showed a slight elevation in 48/48 mice relative to levels in wild type (7/7, 7/0) mice. This difference in outcome most likely reflects increased variance associated with larger group sizes for investigation.

Studies are presently being extended to mice expressing longer CAG repeats (*Hdh*<sup>Q92</sup> and *Hdh*<sup>Q111</sup>), and to later time points. Recent observations by Wheeler et al. (1999) suggest that translocation of huntingtin protein from cytosol to the nucleus, and NII formation, are CAG repeat length-dependent processes. Therefore there is reason to suppose that any energetic changes may vary in time of onset depending on CAG repeat length.

**Objective #2: Measurement of electron transport chain activities in *Hdh* knock-in mice with disease-length and normal-length CAG repeats.**

We used spectrophotometric assays to measure the activities of enzyme complexes I, II-III and IV of the electron transport chain, in homogenate preparations of *Hdh* mouse cerebral forebrain and cerebellum (according to the methods outlined in the proposal). Enzyme activities were measured in 4 month old *Hdh*<sup>Q7</sup> (7/7 CAG repeats), *Hdh*<sup>Q50</sup> (48/7 and 48/48 CAG repeats), and *Hdh*<sup>Q92</sup> (90/7 and 90/90 CAG repeats). Enzyme activities per mg protein were corrected by citrate synthase activity per mg protein, to correct for neuronal/mitochondrial loss. Results are shown in section 11, Appendix 1, Figures 1 and 2. There were no significant alterations in citrate synthase activities in any of the mouse genotypes examined, in forebrain or cerebellum. This indicates no significant loss of neurons and/or mitochondria at this time point. Complex activities were unaltered in *Hdh*<sup>Q92</sup> and *Hdh*<sup>Q50</sup> mice in forebrain homogenates ( $p > 0.05$ ; ANOVA, followed by Fisher's PLSD), although there was evidence of a trend towards reduced complex II-III and IV activities with increasing CAG repeat number (see Appendix 1). In cerebellum homogenates, however, complex II-

III was reduced in 48/7 mice (-38%,  $p < 0.05$ ) and showed a trend to decrease in 90/7 and 90/90 mice (-36% and -24%, respectively). Complex IV activity was also significantly reduced in 48/7 and 90/90 mice (-35% and -42%, respectively;  $p < 0.01$ ), whilst trends towards reduced activity are evident in 48/48 and 90/90 mice (-18 and -31%, respectively). Complex I activity also showed a small, significant reduction in activity in 48/7 mice, relative to levels in wild type mice (-19%;  $p < 0.05$ ), but was unaltered in other genotypes.

These results suggest that there is some degree of impairment of complex II-III and IV activities in *Hdh*<sup>Q92</sup> and *Hdh*<sup>Q50</sup> mice, reaching statistical significance in the cerebellum. It is possible that any region-specific changes occurring in forebrain structures may be being masked by measuring enzyme activities in the whole forebrain. We are currently extending these studies to examine mitochondrial enzyme activities in: a) *Hdh* mice with longer CAG repeats (*Hdh*<sup>Q111</sup>); b) in *Hdh* mice at later timepoints (15 months of age); and c) in more discrete sub-regions of the forebrain.

We have also extended these studies to measure aconitase activities in the same animal population (see Appendix 1). The tri-carboxylic acid (TCA) cycle enzyme aconitase is of particular interest as its' activity has been shown to be markedly reduced in post-mortem tissue from late stage HD patients (Tabrizi et al., 1999), and the same group have also recently reported reduced levels of activity in HD skeletal muscle (Schapira, 1999, unpublished observations). In the present study we found that aconitase activity was significantly elevated ( $p < 0.05$ ) in the cerebellum of 48/48 mice, but was not significantly altered in the cerebellum of *Hdh*<sup>Q92</sup> mice, or in forebrain homogenates of any of the genotypes examined.

**Objective #3: Measurement of ICMR<sub>glc</sub> in the G93A human mutant SOD1 overexpression Tg mouse model of ALS. Analysis of the temporal progression of any glucose use changes by measurement at 60, 90 and 120d of age.**

Transgenic mice overexpressing a human mutant form of SOD1 (a glycine to alanine substitution in exon 4, G93A) develop a disease syndrome whose neuropathology and neurological symptoms closely resemble human ALS (Gurney et al, 1994; Dal Canto and Gurney, 1995). Animals show signs of hind limb weakness at approximately 80-90 days of age, and gradually become paralyzed before dying at 150-180d. Numerous Lewy Body-like inclusions are seen at late stages of the disease. Neuropathological changes including microvesiculation are evident prior to the development of neurological symptoms ( $\approx 70$  days). The involvement of mitochondrial energy metabolism dysfunction in the pathogenesis of this motor disorder is supported by observations that the first pathological events identified in these mice are membrane blebbing and vesiculation of the

mitochondria (Dal Canto and Gurney, 1995). The hypothesis of a gain of function mutant SOD1 is supported by findings that animals expressing high levels of human wild type SOD do not develop the clinical disease, and a recent report that transgene expression levels correspond with the degree of neurotoxicity in mice. The aim of our study was to determine whether there is evidence in this mouse model of FALS of any alterations in energy metabolism prior to the onset of symptoms and mitochondrial morphological changes. We also endeavoured to determine the pattern of glucose use changes over the lifespan of these animals.

In the first year of this study we measured local rates of cerebral glucose use according to the method outlined in the proposal (Browne et al., 1999) in G93A SOD1 mutant mice, and transgenic wild type littermate controls, at 60, 90 and 120 days of age. Some difficulties were encountered in achieving optimal physiological conditions and isotope delivery in this strain of mice. Consequently, we have not included 90 day glucose use values in the results as the group sizes were too small to analyze statistically. In addition, the results reported in Table 3 for glucose use values at 60 and 120 days of age generally reflect group sizes of 2-3, which we consider inadequate for a complete study. Therefore these experiments will be repeated in the second year of this project. However, despite the small group sizes, a marked reduction in glucose use is evident in frontal cortex, motor cortex and striatum of G93A SOD1 mice at 60 days, relative to levels in wild type littermates. This pattern of glucose use reduction is still evident at 120 days of age, although transgenic wild type glucose use levels are also decreased at this time point, relative to levels at 60 days. These results suggest that glucose metabolism in the forebrain is impaired at 60 days of age in G93A SOD1 mice, and that this impairment precedes mitochondrial pathology in this FALS model. As noted above, more experiments must be done to confirm this finding. We will also analyze glucose use in the spinal cord at this time point.

Table 3: Local Cerebral Glucose Utilization (ICMR<sub>glc</sub>) in G93A SOD1 transgenic mice: Time course

	60 Day		120 Day	
	Wild Type	SOD1	Wild type	SOD1
Frontal Cortex	79 ± 3	56 ± 1 **	63 ± 11	36 ± 1 †
Motor Cortex	78 ± 5	60 ± 1 †	68 ± 26	35 ± 1
Striatum	93 ± 4	71 ± 5 *	75 ± 5	45 ± 1 **
Hippocampus CA3	64 ± 18	31 ± 2	59 ± 6	51 ± 1
Cerebellum Grey	43	24 ± 1	41 ± 1	40 ± 2

Local cerebral metabolic rates for glucose (nmol/100g/min) in G93A transgenic SOD1 mutant mice (SOD1; n=2-3 per group) and G93A transgenic wild type littermate controls (Wild Type; n=2-3 per group; except cerebellum at 60 d, n = 1). Data presented as mean  $\pm$  SEM.

\*  $p < 0.05$ , \*\*  $p < 0.01$ , †  $p = 0.05$ , significant difference between SOD1 mutant mice and wild type littermate controls (Student's unpaired t-test).

**Objective #4. Measurement of the temporal profiles of electron transport chain activities in the G93A FALS Tg mouse model of FALS**

We augmented the glucose use studies by investigating mitochondrial respiratory transport chain activities and SOD levels in pre-symptomatic (60 day old) G93A SOD1 mice, using spectrophotometric assays. We compared enzyme activities in forebrain homogenates with levels in transgenic wild type littermate controls (Tg-Wt) and non-Tg wild type controls (Wt). Enzyme activities were corrected for citrate synthase activity (CS), as a measure of mitochondrial content. Complex I activity was significantly increased in G93A SOD1 mice relative to Tg-Wt littermates ( $162 \pm 6$  vs  $130 \pm 7$  nmol/min/mg protein/CS, respectively; mean  $\pm$  SEM;  $p < 0.05$ , ANOVA and Fisher's PLSD). Both Tg-Wt and G93A SOD1 mice expressed equivalent levels of cytosolic SOD1 activity ( $9.4 \pm 0.3$  vs.  $11.4 \pm 0.4$  Units/mg protein, respectively), which were significantly higher than SOD1 levels in non-Tg Wt mice ( $5.3 \pm 0.6$  Units/mg protein;  $p < 0.05$ ). These findings suggest that the metabolic defect is associated with the gene defect and is not attributable to overexpression of human SOD1. There were no significant alterations in complexes II-III and IV activities in the G93A mice, relative to Tg-Wt control levels.

We have also measured electron transport enzyme activities in 100-104 day-old G93A mice (see Table 4). The pattern of elevated complex I activity with no changes in complexes II-III and IV is conserved at this time point. We are currently measuring enzyme activities in 120 day-old animals.

The observation of elevated complex I activity in G93A SOD1 mice is consistent with our previous findings of increased complex I activity in cortical regions of FALS patients with the A4V SOD1 mutation. Results suggest that metabolic defects may exist before signs of motor impairment (about 90 days) and pathological abnormalities (about 70 days).

Table 4: Mitochondrial Respiratory Enzyme and SOD Activities in SOD1 Transgenic Mice:100-104days

	n	Complex I	Complex II-III	Complex IV	Cu/Zn SOD
Wild Type	7	293 $\pm$ 22	1.04 $\pm$ 0.04	1.14 $\pm$ 0.15	4.2 $\pm$ 0.1
Tg-Wild Type	7	354 $\pm$ 28	1.08 $\pm$ 0.09	1.38 $\pm$ 0.14	12.4 $\pm$ 0.8
Tg-G93A	7	386 $\pm$ 6 *	1.18 $\pm$ 0.12	1.48 $\pm$ 0.19	13.4 $\pm$ 0.49

Data presented as mean  $\pm$  SEM citrate synthase (CS)-corrected complex activities (nmol/min/mg protein/CS) and Cu/Zn SOD activity (U activity/mg protein) in cortical mitochondrial fractions and cytosol, respectively. Subjects were transgenic (Tg)-G93A mice overexpressing human mutant SOD1, Tg-wild type littermates, and wild type control mice (age range: 97-108d). \*  $p < 0.05$ , significant difference relative to wild type control mice (ANOVA, and post-hoc Fisher PLSD).

## 7 KEY RESEARCH ACCOMPLISHMENTS

- (i) The finding that cerebral glucose use is not significantly altered in *Hdh*<sup>Q50</sup> CAG knock-in mice at 4 months of age, relative to levels in wild type animals; and that no gene dosage effect is seen in *Hdh*<sup>Q50</sup> mice (48/48 vs. 48/7 CAG repeats).
- (ii) The finding of impaired activities of complexes II-III and IV of the electron transport chain in *Hdh*<sup>Q50</sup> and *Hdh*<sup>Q92</sup> mouse brain cerebellum at 4 months of age (preceding symptom onset and NII formation).
- (iii) The finding that aconitase activity is increased in the cerebellum of *Hdh*<sup>Q50</sup> (48/48) mice at 4 months of age.
- (iv) The finding that cerebral glucose use is reduced in several forebrain regions in the G93A transgenic mouse model of FALS at 60 days of age – a time point preceding the onset of the first pathological changes in these mice.
- (v) The finding of increased complex I activity in the forebrain of G93A mice at 60 days of age, indicating impaired mitochondrial energy metabolism consistent with the defect seen in FALS A4V patients with a SOD1 mutation, which precedes onset of symptoms and pathological changes.

## 8 REPORTABLE OUTCOMES

Browne SE, Andreassen OA, Hughes DB, Ferrante RJ, Jenkins BG, Beal MF. Cerebral energetic defects in transgenic animal models of Huntington's disease. *World Federation on Neurology Research Group on Huntington's Disease*. (1999) 18: 46.

Browne SE, Licata SC, Beal MF. Energetic defects in a transgenic mouse model of familial ALS. *J. Neurochem.* (1999) S27B.

## 9 CONCLUSIONS

We have made substantial progress in characterizing the nature of any changes in cerebral energy metabolism seen in the *Hdh* knock-in mouse model of HD, both *in vivo* and *in vitro*. The next stage of studies in these animals will give insight into the temporal progression of any changes, with regard to the onset of phenotype in these mice, and to the effect of increased CAG repeat number on cerebral metabolic changes.

We have also shown that cerebral metabolism is impaired in the G93A transgenic mouse model of FALS, overexpressing human mutant SOD1, at 60 days of age. This time point precedes the onset of the first pathological changes (70-80 days) and symptom onset (approximately 90 days) in these mice. Our observations suggest that energetic dysfunction may play an intrinsic role in the pathogenesis of the motor neuron disorder seen in G93A SOD1 mutant mice.

The studies reported here represent experimental paradigms in which the efficacies of putative metabolism-enhancing treatments may be tested in future.

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## 11 APPENDICES

### APPENDIX 1.

Figures 1 and 2.

#### Figure Legends:

#### **Figure 1a and 2a: TCA Cycle Enzyme Activities in *Hdh*<sup>Q7</sup>, *Hdh*<sup>Q50</sup>, and *Hdh*<sup>Q92</sup> Forebrain and Cerebellum Homogenates**

Data are presented as mean  $\pm$  SEM citrate synthase (CS) activity (nmol/min/mg protein) and aconitase activity (U activity/mg protein) in forebrain (1b) and cerebellum (2b) homogenates. Subjects were 4 month-old CAG knock-in mice, expressing wild type (7/7) or mutant (48/7, 48/48, 90/7, 90/90) length CAG repeats in huntingtin protein. \*  $p < 0.05$ , significant difference relative to wild type control mice (ANOVA, and post-hoc Fisher PLSD).

#### **Figure 1b and 2b: Mitochondrial Respiratory Enzyme Activities in *Hdh*<sup>Q7</sup>, *Hdh*<sup>Q50</sup>, and *Hdh*<sup>Q92</sup> Forebrain and Cerebellum Homogenates**

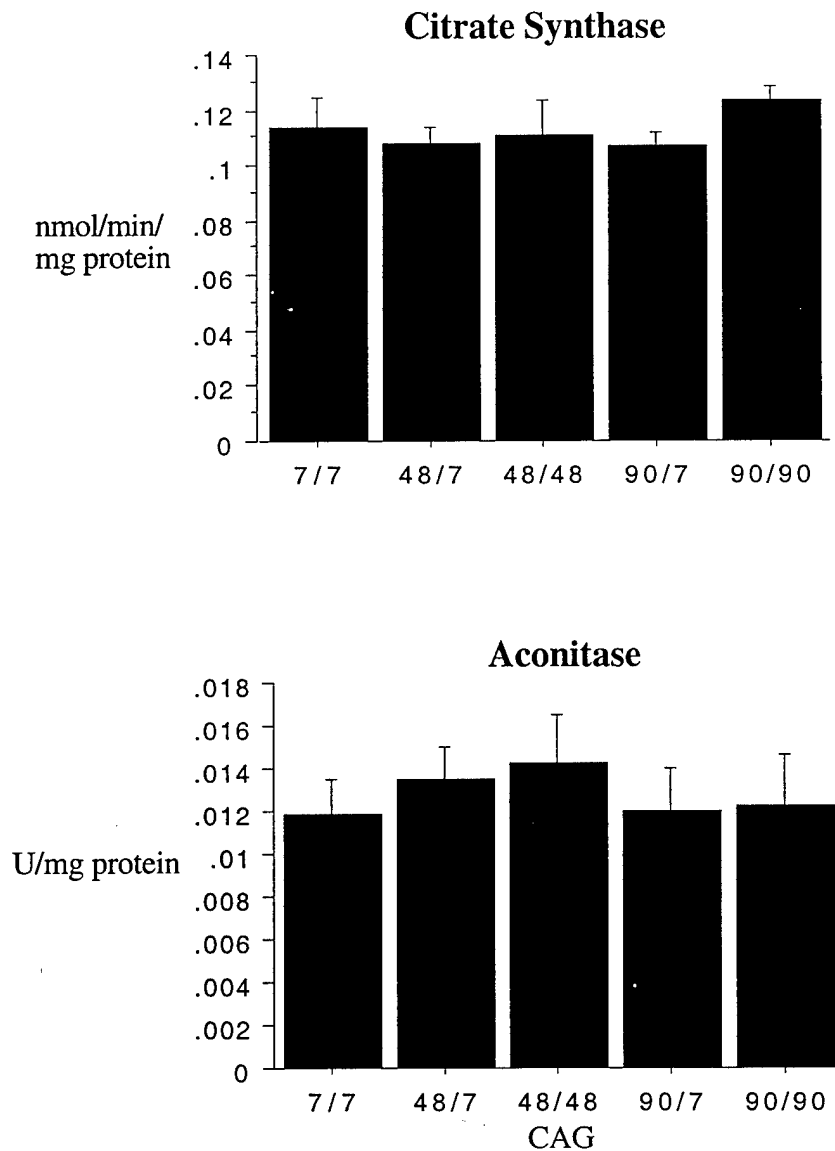
Data are presented as mean  $\pm$  SEM citrate synthase (CS)-corrected complex activities (nmol/min/mg protein/CS) in forebrain (1b) and cerebellum (2b) homogenates. Subjects were 4 month-old CAG knock-in mice, expressing wild type (7/7) or mutant (48/7, 48/48, 90/7, 90/90) length CAG repeats in huntingtin protein. \*  $p < 0.05$ , significant difference relative to wild type control mice (ANOVA, and post-hoc Fisher PLSD).

### APPENDIX 2.

1 copy of each of the abstracts cited in reportable outcomes.

**FIGURE 1a**

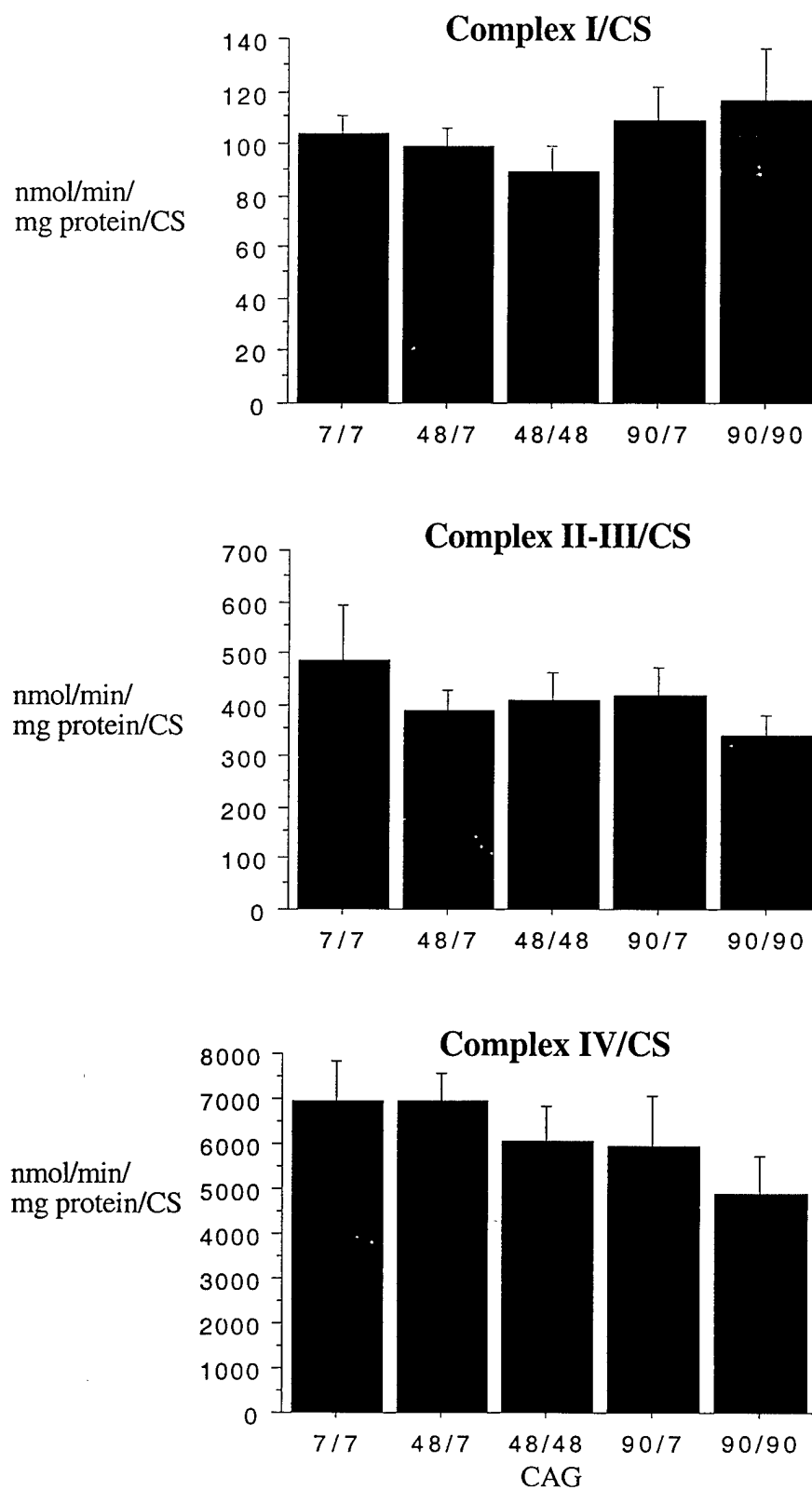
*Hdh* Knock-in Mice  
Forebrain Homogenates



Susan Browne

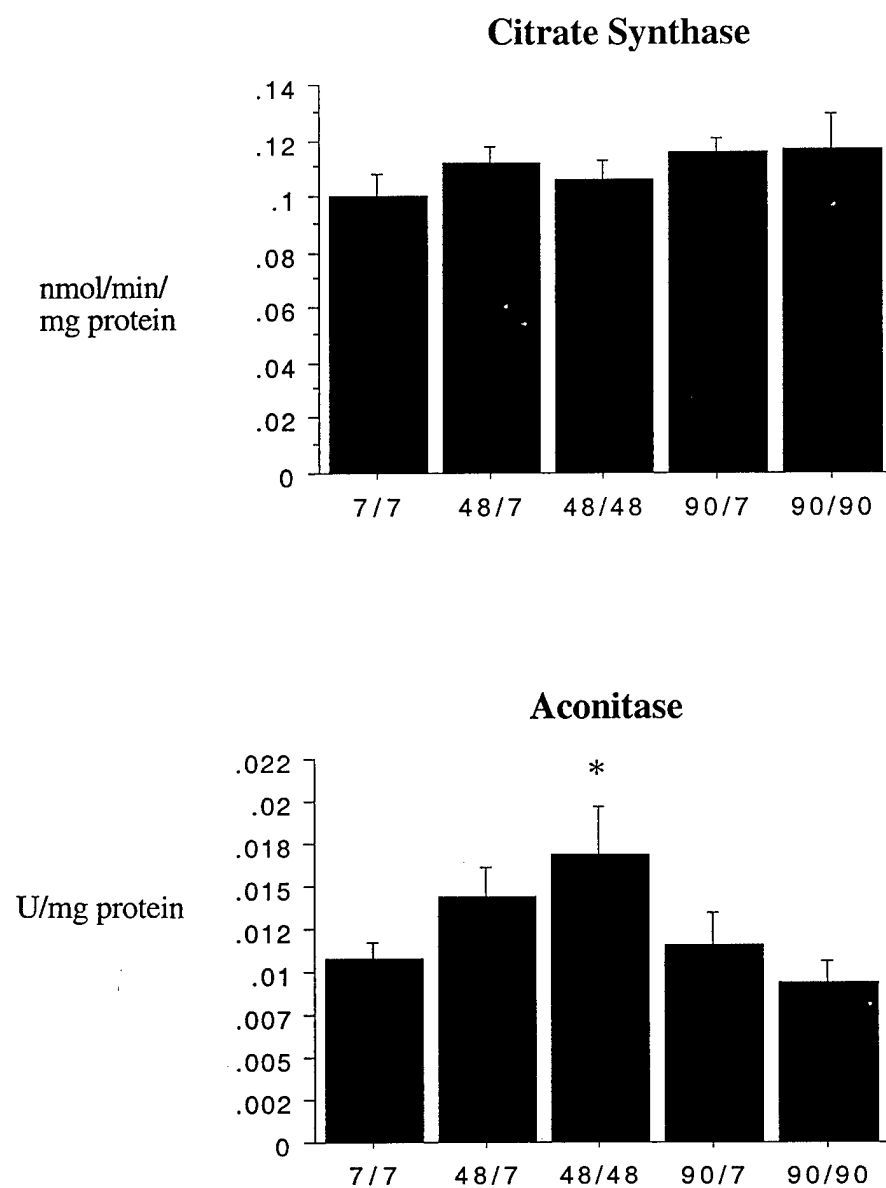
**FIGURE 1b**

*Hdh* Knock-in Mice  
Forebrain Homogenates



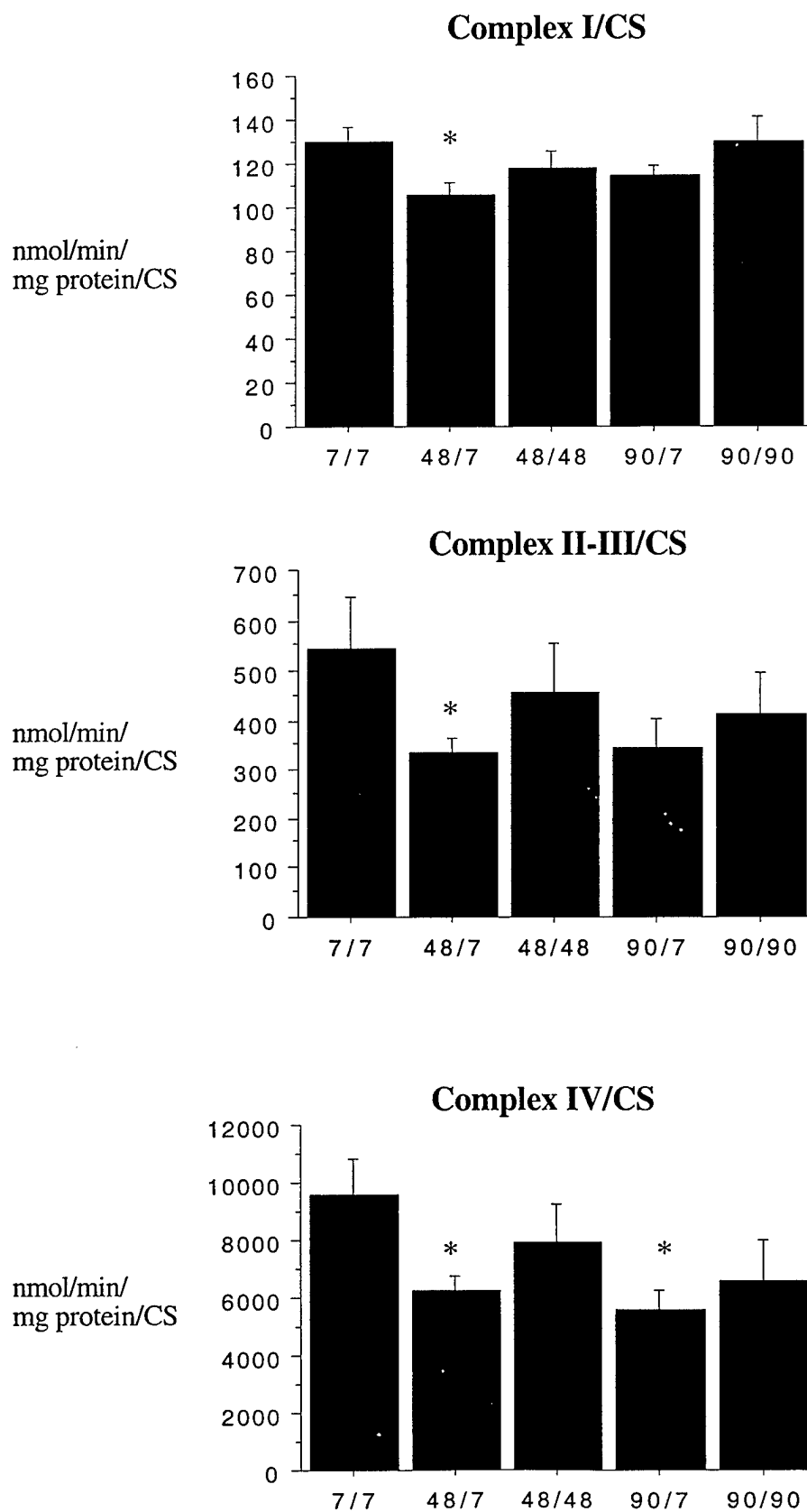
**FIGURE 2a**

*Hdh* Knock-in Mice  
Cerebellum Homogenates



**FIGURE 2b**

*Hdh* Knock-in Mice  
Cerebellum Homogenates



## A

## PHOSPHORYLATION OF TAU PROTEINS AT S262 AND S356 ARE NECESSARY FOR OUTGROWTH OF PROCESSES IN SF9 CELLS

J. Biernat and E.-M. Mandelkow, Max-Planck-Unit for Struct. Mol. Biol., Notkestr. 85, 22607 Hamburg, Germany.

Tau, a neuronal microtubule-associated protein, binds to microtubules and stabilizes them. Binding and stabilization are affected by phosphorylation of tau in different ways. Phosphorylation of Ser262 in the repeat domain by the kinase p110/MARK abrogates binding while phosphorylation of the domains flanking the repeats by proline-directed kinases (e.g. GSK3, cdk5, or MAP kinase) modulates it; both types of phosphorylation are enhanced in the pathological conditions of Alzheimer's disease. We have transfected several isoforms of tau protein in insect cells and observed the outgrowth of processes. This method, explored by Kosik and colleagues, is a sensitive assay for the stabilization of microtubules in cells (for review see Kosik & McConlogue, Cell Mot. Cytoskel. 28:195, 1994). We correlated process outgrowth with tau expression and phosphorylation, as measured by Western blotting, phosphopeptide 2D mapping and sequencing. Insect cells show a complex pattern of P-sites in tau which is similar to the pattern displayed by endogenous tau in the human neuroblastoma cell line LAN-5. We show that mutations of phosphorylatable residues in the repeat domain and in the flanking regions result in opposite effects. Phosphorylation at the KXGS motifs (S262, S356) in the repeat domain is necessary for process outgrowth, whereas phosphorylation at SP or TP motifs in the flanking domains is inhibitory (Biernat et al., Mol. Biol. Cell 1999, in press). - Supported by Deutsche Forschungsgemeinschaft.

## B

## ENERGETIC DEFECTS IN A TRANSGENIC MOUSE MODEL OF FAMILIAL ALS. Browne SE, Licata SC, Beal MF.

Imaging and biochemical studies suggest that energy metabolism is impaired in vulnerable CNS regions in amyotrophic lateral sclerosis (ALS). 10-15% of familial ALS (FALS) patients also express a defect in Cu/Zn superoxide dismutase (SOD1) activity. Impaired free radical scavenging by SOD may result in cell death via metabolic dysfunction induced by increased oxidative damage to mitochondria. Alternatively, findings that G93A transgenic (Tg) mice overexpressing human mutant SOD1 develop a motor disorder resembling FALS suggest that cell death is due to gain of an adverse function associated with SOD1 mutations. To determine if metabolic defects underlie cell death in ALS we investigated the temporal profile of functional metabolic processes in the G93A Tg mouse model of FALS. We measured respiratory transport chain enzyme activities and SOD levels using spectrophotometric assays in pre-symptomatic 60 day-old G93A Tg mice. We found increased complex I activity in G93A mouse forebrain, relative to levels in Tg and non-Tg wild type controls. This is consistent with findings of elevated cortical complex I activity in FALS subjects with the A4V SOD1 mutation. We then used [<sup>14</sup>C]-2-deoxyglucose *in vivo* autoradiography to measure local cerebral glucose use in 60, 90 and 120 day-old G93A Tg mice; ages chosen to correspond with time points preceding the onset of pathological changes (about 70 days), motor impairment (about 90 days), and death (about 130 days). Glucose use was markedly reduced in the spinal cord of 120 day-old G93A Tg mice, relative to Tg and non-Tg wild type control levels. Results suggest that metabolic defects may precede symptom onset in this Tg mouse model of FALS, and imply that drug strategies aimed at ameliorating metabolic defects may be useful ALS treatments.

## C

## AMYLOID BETA PROTEIN AND LIPID METABOLISM: A MISSING LINK IN ALZHEIMER'S PUZZLE?

A.R.Koudinov, T.T.Berezov, N.V.Koudinova. Neurobiology, Weizmann Institute, Rehovot, 76100, Israel, Russian Academy of Medical Sciences, Moscow, Russia, koudin@imb.imb.ac.ru

Amyloid  $\beta$  (A $\beta$ ) is a major constituent of Alzheimer's and Down's syndrome brain amyloid and a normal soluble human protein (sA $\beta$ ). We showed previously that sA $\beta$  in both normal plasma, CSF and hepatic cell culture supernatant is associated with the HDL. These and other facts prompted us to ascertain whether there is a function of A $\beta$  peptide related to cholesterol metabolism and whether it affects cellular lipid synthesis. For this purpose we tested the effect of synthetic A $\beta$ 1-40 and A $\beta$ 1-28 on plasma cholesterol esterification rate. Both peptides at a physiologic concentration of 1 ng/ml similarly inhibited plasma cholesterol esterification rate to 40-50 % of control value. We also tested the effect of A $\beta$ 1-40, homologous to the major circulatory and HDL associated species of Alzheimer's sA $\beta$ , on lipid biosynthesis in human HepG2 hepatoma cells. This culture synthesizes various lipids from [<sup>14</sup>C]-acetate as a precursor. Treatment of cells with different concentrations of A $\beta$ 1-40 decreased the syntheses of various radiolabeled lipid species. The decrease reached saturation at the concentration 10 ng of A $\beta$  per ml of media. The lipids whose synthesis was decreased most were free and esterified cholesterol and phospholipids (25-40 % maximum inhibition). Synthesis of triacylglycerols was also reduced but to a lower extent. Our data suggest that A $\beta$  has extended functions in lipid metabolism. In addition, the observed effects may be of special importance in pathological condition, and contribute to the neurodegeneration, in Alzheimer's disease and related disorders. Supported by The Sir Charles Clore fellowship and Senetek, PLC.

## D

BIOCHEMICAL CHARACTERIZATION OF PARKINSON'S DISEASE (PD) ASSOCIATED  $\alpha$ -SYNUCLEIN

Kahle, P. J., Okochi, M., and Haass, C. Central Institute for Mental Health, Dept. Mol. Biol., J5, D-68159 Mannheim, Germany.

Two independent mutations in the  $\alpha$ -synuclein ( $\alpha$ SYN) gene have been recently identified in kindreds affected by familial PD. Moreover,  $\alpha$ SYN may be the precursor of the non-amyloid component of Alzheimer's disease plaques. Very little is known about the physiological function of  $\alpha$ SYN. The synucleins are developmentally up-regulated after birth.  $\alpha$ SYN and the closely related  $\beta$ SYN are abundantly expressed in brain, while early expression of  $\gamma$ SYN (also called persyn) is widespread in peripheral tissues. Immunohistochemistry had suggested a presynaptic localization of  $\alpha$ SYN in gray matter. Association of recombinant  $\alpha$ SYN with membranes has been shown *in vitro*. However, the nature of  $\alpha$ SYN-associated vesicles remains to be demonstrated. We have raised polyclonal antibodies against  $\alpha$ SYN, which detect the ~19kDa band on Western immunoblots. Using these reagents we show that association of  $\alpha$ SYN with vesicles in post-mortem human brain was labile, and could not be enhanced under coatomer-stabilizing conditions. However, a portion of  $\alpha$ SYN was found in the crude vesicle fraction from rapidly processed mouse brain. We are currently characterizing the  $\alpha$ SYN-associated vesicles with immunological methods. We have also established cell lines stably transfected with wild-type and mutant  $\alpha$ SYN. In these cell lines,  $\alpha$ SYN was expressed as constitutively phosphorylated protein. Phosphatase inhibitor treatment drastically increased the ratio of phosphoprotein, indicating that  $\alpha$ SYN phosphorylation is regulated *in vivo*. We are currently analyzing the  $\alpha$ SYN phosphorylation site(s) and potential kinase(s). These findings may be important for pathological function of  $\alpha$ SYN in PD.

18th International Meeting of the World  
Federation of Neurology Research Group on  
Huntington Disease  
28-31 August 1999

&

13th International Meeting of the  
International Huntington Association  
28 August - 2 September 1999

## CEREBRAL ENERGETIC DEFECTS IN TRANSGENIC ANIMAL MODELS OF HUNTINGTON'S DISEASE

S.E. Browne,<sup>1</sup> O.A. Andreassen<sup>1</sup>, D.B. Hughes<sup>1</sup>, R.J. Ferrante<sup>2</sup>, B.G. Jenkins<sup>3</sup>, M.F. Beal<sup>1</sup>

<sup>1</sup>Department of Neurology, New York Hospital and Cornell University Medical College, New York NY; <sup>2</sup>Bedford VA Medical Center, Bedford MA, and Boston University School of Medicine, Boston MA;

<sup>3</sup>Department of Radiology, MGH-NMR Center, Massachusetts General Hospital and Harvard Medical School, Charlestown MA; USA.

Bioenergetic abnormalities occur in Huntington's disease (HD), but their role in disease pathogenesis is unclear. The development of transgenic mice expressing the huntingtin mutation has facilitated investigation of energetic abnormalities associated with the gene mutation. Several different transgenic mouse strains have been developed to date, differentiated by the site of mutant gene incorporation, CAG repeat length, copy number, and promoter used. These differences are reflected in the phenotypes of the mice generated. We investigated several components of cerebral energy metabolism in two different transgenic models of HD, R6/2 and HdH mice, using metabolic enzyme assays, nuclear magnetic resonance (NMR) spectroscopy, and [<sup>14</sup>C]-2-deoxyglucose (2-DG) in vivo autoradiography. The R6/2 mice (Bates and colleagues) have a CAG repeat length of 141, corresponding with a phenotype similar to juvenile onset HD. Mice exhibit profound symptomatology, including weight loss (onset 6 weeks), motor disorders (8 weeks), premature death (17 weeks), and develop nuclear inclusion bodies at 3-4 weeks of age. Using NMR spectroscopy to measure metabolite levels in vivo, we found that N-acetyl aspartate (NAA) levels decreased exponentially over time (up to 53%) from 6 weeks of age. Since NAA levels reflect neuronal health, and no neuronal death was evident post mortem, these findings suggest a temporal progression of neuronal dysfunction in R6/2 mice. Profound decreases in cerebral levels of glutamate (24%) and succinate (47%), and increases in glutamine (100%), taurine (95%), and choline (76%) also indicate metabolic defects. Furthermore, brain glucose levels were markedly increased (600%) consistent with a diabetic profile, confirmed by findings of abnormally elevated basal blood glucose levels at 10 weeks, and significantly impaired glucose tolerance from 8 weeks of age. Biochemical assays showed elevated

activities of complexes I and II-III of the electron transport chain in R6/2 mouse forebrain at 12 weeks of age, but not at earlier timepoints.

Using the 2-DG procedure to measure glucose metabolic rates in HdH mice (MacDonald and colleagues), we found evidence of abnormal glucose metabolism in mutant mice expressing 48 CAG repeats at 4 months of age, in the absence of any pathological or symptomatic changes. These studies have now been extended to mice expressing longer CAG repeat lengths (90 and 111), and to assessment of mitochondrial metabolic enzyme activities. Results from these studies will further characterize the phenotypes associated with different HD transgenic mouse models, and may lead to the development of therapeutic approaches.